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OXIDIZING ENZYMES AND THEIR RELATION TO "SAP STAIN" IN LUMBER[1]

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The discoloration of sap wood, or "sap stain" as it is commonly called, involves the loss or the depreciation in value of large quantities of lumber annually. Therefore, with the continually increasing value of lumber and the necessity for greater economy in the utilization of wood, the problem of saving and using the sap wood as well as the heart wood of the tree increases in importance each year. In endeavoring to prevent the discoloration of sap wood it is of fundamental importance to discover what agency or agencies produce the stain, and to study their mode of activity.

So far as the writer has been able to determine, there are in general two agencies which produce the discoloration of sap wood. One of these, which is of frequent occurrence and of great economic importance, is a purely chemical reaction which takes place in green sap lumber upon exposure to the air (oxygen). This chemical discoloration occurs in many varieties of wood, and is well illustrated by the reddish yellow or rusty colored sap stain which occurs in the sap wood of alder, birch, and cherry, and by the blue colored sap stain which occurs in the sap wood of red gum (*Liquidambar*). When the freshly cut surfaces of these woods are exposed to the air, under favorable conditions of temperature and moisture, a chemical reaction starts which with varying rapidity produces a colored substance in the wood. Favorable conditions for sap-staining are found during warm weather, and optimum conditions during extremely hot, humid, summer weather, when lumber becomes discolored within a few hours. The examination of microscopic sections of this sap-stained lumber reveals the fact that the colored substance, produced by the chemical reaction, is most conspicuously developed in the wood rays and wood parenchyma cells, living tissues which are largely concerned in the storage and conduction of food in the wood. The second agency which produces discoloration in sap wood is the activity of fungi, which find an abundant food supply in the sap wood, and under favorable conditions of heat and moisture (hot, humid weather) develop with great rapidity. Thus part at least of the blue colored sap stain which occurs so frequently in pine may be produced by the dark colored mycelium of a fungus which is found in the food-containing wood rays and the parenchyma cells surrounding the resin canals. In fact, in all samples of blue sap pine which have come under my observation the color has been produced by this dark colored mycelium. However, it seems very likely that a large part of the blue sap stain in pine is produced by chemical reactions, for I am informed by lumbermen that sap pine lumber frequently stains badly during a few hours. The rapidity with which the discoloration is produced indicates the activity of a chemical reaction.

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[1] Contributions from the Phanerogamic Laboratories of Harvard University. No. 26.

Although the two agencies producing sap stain are so fundamentally different, yet the conditions which favor their activity are very similar. As has been indicated above, each agency, in its activity, is closely related to the food substances contained in the wood. This is shown by the fact that the discolorations, produced by the activity of fungi and by chemical reaction, are most conspicuously developed in the wood rays and wood parenchyma cells. Both agencies producing sap stain are, in addition, dependent upon certain quantities of oxygen (air), heat, and moisture. Thus optimum conditions for sap-staining are found in green sap lumber during hot, humid weather, whereas unfavorable conditions are found in cold, dry weather and in logs immersed in water. It is well known that certain quantities of food, oxygen, moisture, and high temperature are necessary for the rapid development of fungi, but it seems advantageous to examine with greater care a chemical reaction which is dependent upon similar conditions.

Oxidizing enzymes

It has been known for many years that certain soluble ferments, which facilitate the oxidation of organic compounds, are widely distributed in plants and animals. These oxidizing enzymes are of fundamental importance in the nutrition and growth of living organisms. Furthermore, it has been clearly demonstrated that these enzymes produce certain striking postmortem changes in animal and plant tissues. I refer in particular to the postmortem discolorations of certain organic compounds which are produced by the oxidizing activity of these enzymes. The most striking illustration of this oxidation and discoloration in plants was pointed out, in 1883, by a Japanese, HIKOROKURO YASHIDA, who discovered that the formation of black varnish by the oxidation of the latex of the lac tree was produced by the intervention of an oxidizing ferment. Since this discovery, the activity of oxidizing enzymes has been carefully studied in the discolorations which are produced by them in the extracted juices of fruits, vegetables, cereals, mushrooms, and other soft plant tissues. These investigations have shown conclusively that, as in the case of the oxidation of the latex of the lac tree, postmortem discolorations are produced in these juices by the intervention of oxidizing ferments. This change of color which is produced in the extracts of plant tissues has been used as the basis for very delicate tests for oxidizing ferments. A strong blue color produced in an alcoholic tincture of guaiacum, in the presence of oxygen or hydrogen peroxid, indicates the presence of oxidizing enzymes. Upon the basis of these tests certain authorities divide oxidizing ferments into oxidases and peroxidases, according to whether the blue color produced in the tincture of guaiacum occurs in the presence of oxygen or hydrogen peroxid.

It is of interest to note the behavior of oxidizing ferments under variations of temperature. Investigation has shown that optimum temperatures exist for oxidizing enzymes at which they react with great activity. Below these optimum temperatures their activity decreases, and similarly in increasing the temperature above the optimum their activity decreases, and in almost every case their activity is entirely destroyed before a temperature of 100 C. is attained. The activity of oxidizing ferments is also decreased or destroyed by certain antiseptics, and by other chemical substances.

From this consideration of certain properties of oxidizing enzymes, we see that there exists a strong similarity between their oxidizing activity and the chemical reactions which produce sap stain. In each case postmortem oxidization with change of color is produced by solutions in contact with the air (oxygen), and variations in temperature produce similar variations in the activity of the discoloring agency.

Prevention of sap stain

The fact that the temperature of boiling water destroys the activity of oxidases and peroxidases suggested to the writer the idea, that if sap stain were produced through the action of oxidizing enzymes, a simple method of preventing the discoloration of sap wood might be secured by immersing lumber in boiling water. During the spring of the present year the writer has tested this method on small boards (1″×3″×6″) of *Alnus incana* Moench., *Betula populifolia* Marsh., *B. papyrifera* Marsh., and various rosaceous trees. These species were selected as, at this latitude (Massachusetts), the sap wood stains very easily and rapidly during the spring upon exposure to the air, whereas the sap wood of such trees as sweet gum, maple, and basswood stain deeply during extremely hot and humid summer weather. The result of these tests has been to show that boards immersed in boiling water for a few minutes remain permanently unchanged in color, whereas untreated pieces stain rapidly and deeply. The fact that the unboiled boards stained on exposure to the air and that the discoloration was prevented by boiling offered strong evidence for believing that sap stain results from the action of oxidizing ferments. On testing boiled and unboiled boards with the guaiacum tests, the blue color produced in the unboiled boards demonstrated the presence of oxidizing enzymes.

In conducting these experiments the boards which were to be boiled and those which were to serve as controls were in all cases cut from the same part of the same tree, and were subsequently exposed to the same atmospheric conditions of temperature and humidity. The extreme sensitiveness of oxidizing enzymes in their oxidizing activity was clearly demonstrated by the species of wood used in these tests. For example, alder and birch, which in hot, humid weather stain in a few minutes to a reddish yellow or rusty color, stain more slowly at lower temperatures, and in cold dry weather stain but slightly even after an interval of several weeks. In a similar way the depth to which the stain penetrates into the wood varies with temperature and moisture. Boards dried in sheds stain superficially, whereas boards dried in damp conditions in the open stain very deeply and densely. Boards treated in hot water and dried under cover remain unchanged in color throughout, and those placed in the most unfavorable conditions of abundant moisture and high temperature in the open, although they scorch superficially, as does all lumber exposed to the direct rays of the sun, remain unstained beneath this thin layer of tan.

Practicability of hot water treatment

It will occur to some readers that the activity of oxidizing enzymes could be prevented by kiln drying or steaming the wood, and that one of these methods would be more practicable than immersion in boiling water. However, to those familiar with saw mill practice it is evident that in mills sawing many thousand board feet of lumber each day and night it is usually difficult to steam or kiln dry more than a small percentage of the cut. Steaming or kiln drying are expensive processes, and the latter can only be applied to certain varieties and grades of wood. Furthermore, these processes are time consuming and require much handling of the lumber. Some method must be devised for preventing sap stain which can be carried out very cheaply and rapidly, in order not to interfere with the movement of the lumber from the saw to the yard. In other words, the lumber must be removed, treated, and piled as rapidly as it is sawed, in order not to interfere with the daily output of the mill. Some of the larger mills in the south have installed tanks containing chemical substances which destroy the action of oxidases and prevent discoloration by fungi. The lumber as it comes from the saw is carried through this tank upon carrier chains and thus immersed and coated with the chemicals. Certain difficulties have been encountered in the use of this method. One of the most serious is the fact that the chemicals used fire proof the outer surface of the boards, and when the treated lumber is shipped to northern markets for finishing the planings and shavings cannot be burned. This obliges the use of coal to run the planers and other machinery, and the non-combustible planings must be disposed of.

In the treatment of lumber with boiling water a similar method could be employed. Long shallow tanks in which water is heated by steam pipes or by steam exhausts could be arranged so that the lumber from the saw could be passed through the tanks on carrier chains.

During the coming summer the writer will extend his experiments to include a wider variety of woods, and will conduct experiments of a practical nature in a large southern saw mill, to determine the relative advantages of hot water treatment and treatment with chemical substances.

Summary and conclusions

1. Sap stain is in general produced in two ways, by the attacks of fungi and by chemical discoloration.
2. Chemical discoloration is produced in sap wood by the activity of oxidizing enzymes.
3. Hot, humid weather is very favorable to the activity of these ferments, and cold winter weather is unfavorable.
4. Oxidizing enzymes which produce sap stain in wood are destroyed and their oxidizing action prevented by a temperature of 100° C.
5. Treating the sap wood of alder, birch, and cherry with boiling water destroys the oxidizing enzymes in the wood and prevents sap stain.
6. Treating sap lumber in long tanks of boiling water appears to be a practical method of preventing sap stain, and to be well adapted to saw mill practice.

In conclusion the writer wishes to express his sincere thanks to Professor E. C. JEFFREY for valuable assistance in carrying out this investigation, to Professor G. E. OSTERHOUT for advice and suggestions, and to MR. W. R. BUTLER for material of sap-stained lumber.

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